Emir. J. Food Agric. 2011. 23 (5): 452-459
http://ejfa.info/

Development of field technique for mass screening of chickpea (*Cicer arietinum* L.) germ plasm under drought prone environments

M. Yaqoob[1*], Phill A. Hollington[2] and Jahn Gorham[2]

[1]Pulses Program, NARC, Islamabad; [2]CAZS-NR University of Wales, Bangor, United Kingdom

Abstract: In Pakistan more than 95% chickpea production comes from rainfed lands where drought is the major abiotic constraint. Chickpea screening for drought under natural environment is not usually reliable due to risk of uncertain rainfall. Studies for developing germ-plasm screening technique are more reliable under controlled environment. In present investigations a Glass house experiment was conducted at Centre of Arid Zone Studies, CAZS-Natural Resources, University of Wales, Bangor, UK during 2006-07. Four chickpea varieties/lines two each from desi and kabuli group were planted in Bench top raised bed measuring 50 x 50 x 30 cm. The experiment was laid out in RCBD with Split Plot arrangements having six replications. The crop was maintained under various artificial moisture stresses imposed at different plant growth stages. Treatments included well watering (control), moisture stress to 40 days old seedlings (FOS), stress at pre-flowering stage (SPS), stress at flowering stage (SFS) and stress at 25% pod formation stage i.e. terminal drought stress (TDS). The results revealed highly significant variation in yield contributing traits. Among various treatments, the moisture stress at pre-flowering stage (SPS) was found to be more damaging to yield and yield contributing variables in both the types of chickpea with maximum loss of 41.29% in grain yield as compared to control. Besides poor yield, the crop was also harvested late under SPS conditions. The moisture stress to chickpea plant at pre-flowering (SPS) stag was found quite harmful and detrimental, suggesting the more critical stage for germ plasm screening against drought prone environment.

Key words: *Cicer arietinum*, germ plasm, drought

تطوير تقنية حقلية لدراسة الجيرموبلازم لمحصول الحمص (*Cicer arietinum* L.) تحت ظروف الجفاف

م. يعقوب[١]* ، فل أ. هوللينقتون[٢] و جاهن قورهام[٢]

[1]برنامج البقول ، ن أ ر ك، اسلام اباد؛ [٢] ك أ ز س – ن ر جامعة ويلز ، بانقر ، الولايات المتحدة

الملخص:في الباكستان أكثر من ٩٥ ٪ من انتاج الحمص يأتي من الأراضي البعلية حيث تعتبر ظاهرة الجفاف هي العائق الرئيسي للإنتاج. دراسة غربلة الاصناف لمقاومة محصول الحمص للجفاف تحت الظروف الطبيعية لا يعتمد عليه عادة بسبب خطر هطول الأمطار غير المتوقع . دراسات لتطوير الجيرموبلازم هي أكثر موثوقية تحت الظروف المتحكمة بها. أجريت تجربة تحت ظروف البيت الزجاجي في مركز للدراسات المناطق القاحلة، CAZS، الموارد الطبيعية، جامعة ويلز ، بانغور، المملكة المتحدة خلال ٢٠٠٦-٠٧. زرعت أربعة أصناف من محصول الحمص كل صنفين ينتموا الى مجموعة ديسي والصنفين الاخرين الى المجموعة كابولي على مصاطب ذات ابعاد ٥٠ × ٥٠ × ٣٠ سم. استخدم نظام القطع المنشقة RCBD بستة مكررات. وتم تعريض المعاملات الى فترات جفاف مستحدثة وتحاكي المفروضة على النبات بمختلف مراحل النمو. وشملت المعاملات ري جيد (الشاهد)، والإجهاد الرطوبة الى عمر ٤٠ يوما (FOS)، والإجهاد الى مرحلة ما قبل الإزهار (SPS)، والإجهاد الرطوبي الى مرحلة الإزهار (SFS)، والاجهاد الرطوبي الى ٢٥% من مرحلة من تكوين القرون أي إجهاد الجفاف المتأخر (TDS). أظهرت النتائج تفاوتا واضحا بين المعاملات المختلفة من حيث الصفات المساهمة في الانتاج. وجد أن الإجهاد الرطوبي في مرحلة ما قبل الإزهار (SPS) كان أكثر ضررا على المحصول والعوامل التي تؤثر على الانتاج في كل أنواع الحمص مع خسارة قصوى تبلغ ٤١,٢٩ ٪ في محصول الحبوب مقارنة بالشاهد. بالإضافة الى الغلة القليلة، كان حصاد المحصول متأخر في يما يخص معاملة الإجهاد الرطوبي الى مرحلة ما قبل الإزهار (SPS). وجد ان الإجهاد الرطوبي الى مرحلة ما قبل الإزهار (SPS) ضار وقاتل، مما يشير أهمية هذه المرحلة في دراسة الجيرموبلازم للأصناف تحت ظروف للجفاف.

*Corresponding Author, *Email*: yaqoobawan313@gmail.com
Received 23 January 2010; Revised 03 June 2011; Accepted 03 June 2011

Introduction

Chickpea is third pulse crop of the world and mainly cultivated on residual soil moisture (FAO, 2006). In Pakistan, it is also grown on preserved soil moisture under rainfed conditions. In-time rain fall especially during cropping season helps the plant growth and development. Too early precipitation i.e. during July or early August is not desirable as most of the soil moisture is exhausted before crop sowing. This situation creates drought, exerting bad effect on crop. Inadequate water availability restricts the plants to express their full genetic potential. Drought is meteorological event which implies the absence of rainfall for a long period of time enough to cause moisture depletion in soil and moisture deficit with a decreased water potential in plant tissues (Kramer, 1980).

Different strategies are developed to harvest good yield under drought and rainfed conditions. Early crop sowing greatly improves the chickpea production in various ecological zones of the country. The resistance to drought prone environment can be obtained through various mechanisms, including escape, dehydration avoidance or dehydration tolerance (Blum, 1988). Yield potential and drought escape through early flowering are the two major components for drought resistance in chickpea (Silim and Saxena, 1993a). The early flowering varieties due to shorter growth period in field are not necessary always high yielding. The only alternate to increase the chickpea production in various moisture limiting areas is the development of drought tolerant genotypes and efficient germplasm screening methodology for introducing new high yielding genotypes. Screening techniques must be refined efficiently to evaluate the genotypic performance at a critical developmental stage under moisture stress conditions. The research conducted so far in Pakistan has been confined to screening germplasm against drought under fragile field conditions i.e. without imposing moisture stress at specific physiological growth stages of the crop plant. Such types of field screening against drought might be misleading and unreliable. It is, therefore, of dire need to screen out the genotypes under schedules moisture stresses imposed at various crop developmental stages. Different plant and root traits and their pattern are needed to be identified to understand their role towards drought avoidance. The most damaging and crucial moisture stress during specific plant growth stage must be identified for developing criterion for screening chickpea germplasm under given drought conditions. Such studies also help understanding the drought tolerance mechanism and contribution of various traits for grain yield under moisture deficit soils. Some drought tolerance chickpea lines with comparatively higher yield have been identified under moisture fix up conditions (Saxena et al., 1993). The drought tolerance in their case was found to be directly proportional to deep root system and high leaf water potential (LWP). They found 93% higher root dry weight in drought tolerant chickpea lines (ICC 4958) as compared to standard check. Matsui and Singh (2003) and Anbessa and Bejiga (2002) have also selected 18 drought tolerance genotypes among a lot of 482 chickpea lines and reported reduced water loss from plant and extensive extraction of soil moisture as the factors adaptation of drought tolerant genotypes. Matsue and Singh (2003) observed that center of root dry matter and root length density of two cowpea varieties moved downwards significantly, under water stress conditions. In present experiment different chickpea lines were studied by imposing the artificial drought at various plant growth stages under controlled conditions to identify the best time/stage of plant to keep under moisture stress for screening the germplasm on mass scale.

Materials and Methods

The experiment was conducted to develop the most effective and reliable chickpea germ plasm screening technique under drought conditions. For this purpose four chickpea varieties/lines two each from desi and kabuli group were planted in Glass House at Research site of Centre of Arid Zone Studies and Natural Resources, (CAZS-NR) University of Wales, Bangor, United Kingdom during 2006-2007.

Two approved varieties Sheenghar-2000 (desi) and Lawaghar-2000 (kabuli) along with two breeding lines SL-05-03 (desi) and F-97-155C (kabuli) were used in present investigations. The seed of each variety was first germinated in P12 Plug Trays using compost to flee out any risk of germination failure and to have healthy seedlings in main experimental pots. Seven days old seedlings were then transferred to the Bench Top Raised Bed (BTRB) measuring 50 x 50 x 30 cm. Each BTRB comprised of four plants (one representative plant of each variety/line) spaced at proper distance. The experiment was laid out in RCBD with split plot arrangements. There were five treatments (including control) with six replications. All the chickpea varieties/lines were maintained under four different moisture stress treatments imposed at various plant growth stages. Various scheduled moisture stresses were imposed by stopping the water for a period of 30 days in each treatment.

Treatments

- T-1 Well watering (Control)
- T-2 Moisture stress to 40 days old seedlings (FOS)
- T-3 Stress at Pre-flowering stage (SPS)
- T-4 Stress at Flowering stage (SFS)
- T-5 Stress at 25% Pod formation stage i.e. terminal drought stress (TDS).

The moisture stress was terminated as soon as treatments qualify for re-watering after stipulated stress period i.e. 30 days. The non-stressed treatments (control) had been receiving optimal irrigation regularly throughout the cropping period. The glass House temperature was maintained at 18 and 15 ^{0}C for 16 hours (day) and 8 hours (night) respectively. The data were recorded from following plant traits:

1. Days to flowers initiation
2. Plant height (cm)
3. Days to maturity
4. Total number of pods per plant
5. Number of healthy pods per plant
6. Pods abortion %age
7. 10 grain weight (g)
8. Plant biomass (g)
9. Grain yield per plant (g).
10. Harvest index.

Statistical Analysis

The data acquired were subjected to analysis of variance based on RCBD with mean separation by LSD, using SPSS software version 12.0.

Results and Discussion

The results of analysis of variance along with the effect of moisture stress treatment is given in Table 1, whereas, the performance of chickpea varieties for various plant trait are shown in Table 2. The results given in Table 1 revealed that the traits like, days to maturity, total number of pods, number of healthy pods, number of grains per plant, plant biomass and grain yield were highly significantly affected due to various moisture stresses. The weight of grain was also significantly affected due to stress conditions. Whereas, moisture stress had negative effect on days to flower initiation, plant height, pod set % age, pods abortion % age, and harvest index % age. The main effect of various chickpea genotypes was only significant on days to flowers initiation, plant height, seed weight, number of grains per plant and plant biomass. The interaction effects between different moisture stress and chickpea genotypes were found to be non-significant for all the traits.

Phonology

Flowers initiation in crop plant is a qualitative trait depending upon the genetic makeup of variety. It is also highly influenced by variation in prevailing environments. Moisture stress usually tends crop plant develop early flowers. The flowers initiation in present studies was not significantly affected by various moisture stress treatments. However, chickpea genotypes were highly significantly different in flowers admittance. Days to flowers initiation were not dependent upon the types of chickpea (desi and/or kabuli) in present investigations. The minimum 61 as well as maximum (70) days to flower initiation were taken by kabuli genotypes, while desi type remained intermediate with 62 and 63 days. Kumar et al. (2004) have also found non-significant flowering in chickpea under drought conditions.

Table 1. Mean and level of Significant for various plant traits in chickpea (*Cicer arietinum* L) as influenced by moisture stress treatments.

Stress Treat	Flow NS	Maturity **	Height NS	Total Pod**	Healthy Pod**	Pod. Set.% NS	Pod Aberration %age NS	Plant Biomass **	Grains per Plant*	10GW **	HI% NS	Grain Yield (g) **
Control	65.21	183BA	106.67	59.33A	38.83A	66.29	33.71	71.38A	48.25A	2.26B	18.97	10.51AB
FOS	64.91	186AB	102.79	42.33AB	29.42AB	69.38	30.62	50.88AB	3AB5.46	2.54B	21.68	8.97B
SPS	67.13	188 A	98.71	**30.33B**	**21.58B**	72.05	27.95	**30.43B**	**26.08A**	**2.54B**	33.93	**6.17B**
SFS	62.54	184AB	97.17	47.71AB	31.50AB	68.02	31.98	55.16A	34.71AB	2.48B	18.61	8.41B
TDS	60.96	**181.B**	95.13	58.67A	41.79A	70.63	29.37	51.43AB	47.63	3.08A	29.64	14.48A

* = Significant at 0.5% level, ** = Significant at 0.1% level, NS = Non-significant

Table 2. Means for various plant traits as influenced by various chickpea (*Cicer arietinum* L.) genotypes.

Varieties	Days to Flower initiation **	Plant Height **	Grain yield per plant (g) *	10 seed weight (g) **	Plant Biomass (g) **
Sheenghar-2000	63.C	87.67B	37.40B	2.53B	46.34AB
Sl-05-03	62.B	98.33B	48.23A	**2.46B**	**58.99AB**
Lawaghar-2000	70.A	83.17B	27.20C	3.08A	35.91B
F-97-155C	61B	131.20A	40.87B	2.26B	66.19A

** = significant at 0.1% level, Interaction = Non-significant in all traits.

The Trait showing non- significant variation were omitted in Table-2

Days to Maturity

Crop maturity is the end plant activities resulted in production of seed and biomass. Maturity, being a genetic trait was highly influenced by various stress treatments. The number of days to maturity in present experiment ranged from 181 to 188. The SPS though had delayed maturity by 5 days as compared to control it however, availed, statistically similar period to well watering, FOS and SFS treatments. The maturity was accelerated in terminal drought and crop matured 7 days earlier then SPS while it was statistically similar to well watering (Table 2). It was interestingly noted that various chickpea genotypes had shown non-significant variation in days to maturity while all were highly affected by fluctuation in moisture levels at various growth stages. This showed that maturity is highly influenced by the environmental factors particularly, under abiotic stress conditions. Kanouni et al. (2002) got some early lines with higher grain yield as compared to late maturing lines of chickpea. Meena et al. (2006) have also had greater variation in maturity of chickpea with early to late under drought environment.

Number of pods per plant

There found highly significantly variation in total number of pod (filled and unfilled) due to various stress treatments. The SPS was most damaging to chickpea as it had reduced the total number of pods by 48.88% against well watering. Its effect was however, statistically similar to FOS and SFP. Total number of pods produced by TDS were almost similar to well watering and decreased by only 1.11% (Table 1). The genotypic effect on total number of pods per plant was statistically non-significant (Table 2). The entire variation in total number of pods was attributed to various stress treatments. The trend of healthy pods production under various stress treatments was quite similar to that of total number of pod under moisture stress conditions. The moisture stress at pre-flowering stage (SPS) also remained harmful to pods and produced only 21.58 pods per plant as compared to 38.83 pods under well watering with reduction of 51.86% pods. The TDS was also statistically similar to well watering and produced only 6.78% higher pods then well watering (Table 1).

In case of genotypic contribution, the results showed a non-significant variation among the four (desi and kabuli) lines for number of healthy pods. In present investigation SPS had significantly reduced the total as well as healthy pods with same ratio as compared to other treatments. The pods production in chickpea was also found sensitive to temperature and mostly remained type specific i.e. desi and kabuli types had variable response to temperature. The kabuli type chickpea were found more sensitive to high temperature than desi chickpea. Wang et al. (2006) had also found significant variation in desi and kabuli type chickpea in response to high temperature. The higher temperature stress decreased 42% seeds per plant in kabuli and 35% in desi chickpea. In present studies well watering and TDS had produced higher number of healthy pods and grains as compared to rest of the moisture stress treatments. Desi chickpea produced higher number of grains while the weight of grain remained higher in case of kabuli chickpea. Wang et al. (2006) also confirmed that desi chickpea produced higher pods as well as yield then kabuli type. This was due to the reason that kabuli types are more sensitive to high temperature and drought as compared to des type (Leport et al., 2006). Seed yield in chickpea is associated with phenology which ultimately affects the number of pods and grain yield (Berger et al., 1989). High temperature stress reduces the seed yield in desi as well as kabuli chickpea. While stress at pod filling stage had reduced 46% yield in pigeonpea due to lack of active root system. The Desi type also had higher yield than kabuli type chickpea (Leport et al., 2006; Wang et al., 2006). The plant at this stage might not be able to extract moisture from deeper layer at 60 cm. The moisture stress severity can be overcome partly through remobilization of stored assimilates to pods through escape (Westgate et al., 1989). The grain yield can also be affected under high temperature and drought due to pod abortion especially in kabuli chickpea.

Number of grain per plant

Number of grains per plant were maximum in well watering (48.25) followed by TDS with 47.63 grains per plant. The least number of grains per plant were counted in SPS with 45.95% reduction against control. The SPS was most detrimental and reduced the number of grains per plant (26.08). This again showed the same trend as in case of total and healthy pods per plant. The treatments FOS, SFS and SPS remained statistically similar and produced 35.46, 34.71 and 26.08 grains per plant respectively. Likewise, well watering and TDS produced the highest grains per plant and were statistically at par to each other (Table 1). The number of grains per plant was also significantly differed due to various chickpea genotypes. Desi type again showed insensitivity to moisture stress then kabuli types and showed higher number of grains. Consequently, significantly higher number of grains 48.23 and 40.40 per plant were respectively produced by Sheenghar-2000 and S1-05-03 both belong to desi group. While both kabuli types viz. Lawaghar-2000 and F-97-155C had 30.87 and 27.20 grains per plan respectively (Table 2). The number of seed per plant is important trait having positive impact on grain yield (Yadav et al., 2005), whereas, this trait was non-significantly affected by moisture stress and genotypes (Kumar et al., 2004).

Grain weight (g)

The weight of grains is usually recorded on basis of 1000 seeds or at least 100 seeds, while in present studies; it was recorded from 10 grains keeping in view the quantity of seed produced at minimum level. We found highly significant variability in grain weight due to various moisture stress as well as genotypes. The weight per 10 seeds ranged from 2.42 in SFS to 3.83 in well watering. The size of seed was badly affected at SPS and SFS with seed weight of 2.36 and 2.42 gram per 10 seeds respectively (Table 1). The size of grain reduced in SPS by 38.38% against well watering and 35.69% against TDS respectively. The grain size in FOS, SPS and SFS remained statistically uniform with value of 2.53, 2.36 and 2.42 grams per 10 seeds. In case of genotypic effect, the grain size was also highly significantly influenced by both the types of chickpea. The kabuli type produced significantly heavier seed with 2.96 grams by Lawaghar-2000 and 3.05 grams by F-97-155C per 10 grains. Desi types (Sheenghar-2000 and S1-05-03) showed small seed 2.53 and 2.46 grams per 10 seed respectively (Table 2). Grain yield can be possibly increased through improving the size of seed (Yadav et al., 2005). Seed weight is one of the yield enhancing components in chickpea having direct positive effect on yield (Meena et al., 2006).

Plant Biomass Weight

Plant biomass weight was highly significantly affected by stress treatments as well as chickpea genotypes. The highest plant biomass of 71.38 grams per plant was weighed in well irrigated treatment which was significantly higher than rest of the stresses. The minimum plant biomass was recorded in SPS with 30.43 grams plant^{-1} which was significantly lower than rest of the three stresses. The treatment like FOS, SFS and TDS remained statistically at the same level and produced medium plant canopy with biomass weight of 50.88, 55.16, and 51.43 grams per plant respectively (Table 1). In case of genotypic effect, the highest plant biomass 66.19 grams was recorded in kabuli type F-97-155C which was statistically similar to a desi type S1-05-03 with 58.99 grams. The plant biomass of rest of the two genotypes one each desi (sheenghar-2000) and kabuli (Lawaghar-2000) was also statistically non-significant to S1-05-03 (Table 2). The early moisture stress to chickpea crop had more damaging effect on chickpea then late stress (Leport et al., 2006). The chickpea lines were highly affected due to moisture stress. Bold seeded genotype produces high biomass then small seeded (Kumar et al., 2004).

Grain yield

Grain yield the ultimate aim was highly significantly affected by both the factors of variations (Table 1 and 2). The highest yield of

18.57 gram per plant was recorded in well watering but it was not statistically more than TDS with 17.48 grams per plant. The remaining three stresses were statistically similar and produced very poor yield. The SPS was again severely haunted by the moisture stress and ultimately reduced grin yield up to 41.29% as against control (Table 1). The grain yield was also affected by genotypes as well as type of genotypes (desi and kabuli). The highest yield was produced by desi type. The line S1-05-03 and cultivar sheenghar-2000 (both belong to desi type) had produced 14.16 and 13.25 grams of grain yield per plant. These desi types being significantly at par with each other remained superior to both the kabuli types (Lawaghar-2000 and F-97-155C) which in turn produced lower yields of 9.41 and 8.05 gram per plant respectively. The grain yield was increased mostly due to increase in number of seeds per plant, seed per pod, seed weight and harvest index, therefore, selection of drought tolerance is more reliable under early drought then late in chickpea crop (Meena et al., 2006). The seed yield is also increase due to increased number of branches, pods and harvest index (Kumar et al., 2004).

In the preceding discussion, it can be concluded that chickpea being drought tolerant crop is yet highly sensitive to drought at pre-flowering stage. The plants obtain and retain substantial water in its tissues during development stage i.e. before entering in reproductive phase. This amount of stored water is utilized by the plant during reproductive and grain formation phase if exposes to drought prone conditions. In present investigations we observed pre-flowering stage the most sensitive to drought conditions. It is, suggested that chickpea germplasm may be given moisture stress at this stage for screening against drought.

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